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Effect of earthworm density on the growth and reproduction of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) in culture

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Summary. Experiments examining growth and reproduction of *Lumbricus terrestris*, by varying earthworm density at 20 °C, in laboratory culture, are described. Densities up to 62 g live mass litre^{-1} were attained during growth experiments in 0.3 litre pots, but sexual maturity of the earthworms was not attained above 53 g live mass litre^{-1}. From the hatchling stage minimum time to maturation was twelve weeks reaching a final density of 15 g live mass litre^{-1}. Greatest reproductive output at 4.4 cocoons worm^{-1} month^{-1} was recorded at a density of 15 g live mass litre^{-1} in 0.75 litre pots with a depth of 0.076 m. Cocoon production occurred in 0.6 litre pots but high mortality was recorded above 19 g live mass litre^{-1}. Overall the results suggest that in a large production system soil depth could be kept to a minimum but population densities ought to be maintained towards the lower end of the range 15–25 g live mass litre^{-1}.

Key words: Density, earthworm, reproduction, growth, *Lumbricus terrestris*

Introduction

In temperate soils, in the absence of human intervention, the maintenance of soil structure, fertility and under certain circumstances even pedogenesis itself may be largely attributed to the activities of earthworms. These activities improve soil aeration, drainage, the availability of nutrients for plants and generally integrate soil organic and mineral elements to form aggregates and improve soil structure. *Lumbricus terrestris* L., the largest earthworm in northern Europe, is known for contributing greatly to improved soil status. In the absence of earthworms, soils may deteriorate considerably showing increased bulk density and reduced penetrability, infiltration rate and organic matter content (e.g. Clements et al. 1991). Conversely the addition of earthworms to areas where they were previously absent has resulted in relatively rapid structural improvement for a variety of soil types (Curry 1988). Using earthworms to promote soil amelioration, where appropriate, is now regarded as a step towards complete rehabilitation of degraded soils. The large number of earthworms required for such schemes could be obtained through field-collection (e.g. Tomlin 1983) but the quality of these animals and their chances of survival after broadcast-inoculation is questionable (Butt et al. in press). A viable alternative for the production of sustainable field populations may be mass rearing of the required species under controlled conditions.
Vermiculture of litter dwelling species such as *Eisenia fetida* (Savigny) has been examined in great detail and is reviewed by Lofs-Holmin (1985), but the mass rearing of true soil dwelling species has not. Brun et al. (1987) propose that the intensive production of soil inhabiting species would, if feasible, prove advantageous to soil improvement schemes and

Lee (1992) views research into controlled introductions, to maximise beneficial soil effects, as a necessary development in future earthworm research.
For *L. terrestris* the key environmental/biotic factors; temperature, moisture, nutrition and population density, have all been examined to some degree (Hartenstein & Amico 1983; Lofs-Holmin 1983; Butt 1991; et al. 1992), but certain aspects require further investigation. There is a particular need to establish more clearly an acceptable stocking density and the carrying capacity of the chosen system, if this species is to be cultured intensively.
Experiments with litter dwelling species have confirmed that density dependent effects (Nicholson 1954) play an important part in limiting population growth. For example, Neuhauser et al. (1980) found that growth rates of *E. fetida*, fed activated sludge on soil in 0.3 litre pots, were reduced as the experimental populations were increased from 4 to 16 earthworms per pot. Working with the same species, Tomlin & Miller (1980) showed that mean cocoon production in 0.2 litre pots was also reduced in a density dependent manner. Similarly, Reinecke & Viljoen (1993) showed that *Eudrilus eugeniae* (Kinberg) tended to be smaller and produce fewer cocoons at higher densities despite ample food provision. Less information on population density is available for *L. terrestris*. In Britain this species is naturally dominant in orchards and common in pasture/arable soils, with up to 70 – 75 individuals m^{-2} (Edwards 1983). However in terms of intensive production this number per unit area measurement is of limited use. Experimental volumetric requirements are provided by Evans & Guild (1948), who successfully kept five *L. terrestris* in 3-pint jars but found that cocoon production for the same number was greater in 5-pint jars (2.85 litres). Ashby (1976) found wooden boxes of 35 litres to be convenient for handling and maximum production, whereas Lofs-Holmin (1983) preferred 15 individuals in 20 litre plastic boxes for reproductive studies. Hartenstein & Amino (1983) successfully cultured up to 8 earthworms (31 g live wt) in 1.4 litres during growth examination. More recently Butt et al. (1992) have shown that four mature *L. terrestris* can be maintained, and will reproduce continuously over a twelve month period, within a 2 litre vessel.
During the present study experiments were conducted to assess the lower limits to which volume for successful culture might be taken and to establish optimal stocking densities for the growth and reproduction of *L. terrestris*.

Materials and Methods

Temperature and Culture vessels

All of the experiments were conducted at 20 °C, a temperature greater than that normally experienced in temperate soils (e.g. Lee 1985) but within the range of 15 – 25 °C maintained in experiments with *L. terrestris* by Hartenstein & Amico (1983). At 20 °C *L. terrestris* shows both rapid growth and cocoon development (Butt et al. 1992).
All pots used were made of plastic and had removable lids which were provided with six mounted-needle size air holes. Those used in the first three experiments were obtained from Gregory & Co., London, SE4, U.K., whereas Lakeland plastics, Windermere, Cumbria, U.K. supplied the 'stack-a-boxes' used in experiment four.

Soils and feedstocks

The soil used in the growth experiments was a loamy sand with a pH of 7.5 (Butt 1991), that used in the reproduction experiments was a loam obtained from Boughton Loam Ltd., Kettering, U.K. The latter was pre-sterilised and sieved to less than 6 mm. It had an organic content of 5 percent and a pH of 6.4. Before use soil moisture content was adjusted to 25 – 30% wet soil mass. The feeds used were of two types. Paper pulp, which was obtained from Grovehurst Energy, Sittingbourne, Kent, U.K. and separated cattle solids which were obtained from The Institute of Animal Health, Compton, Berks., U.K.

Laboratory grade yeast extract, used in the second experiment, was produced by Sigma chemical Co., St. Louis, USA (ref. Y-4000). Further details of feedstocks are provided by Butt et al. (1992).

Growth

Experiment 1: Thirty-six immature *L. terrestris*, with a mean mass of 2.1 g, were selected from field-collected earthworms (Raw 1959) which were maintained in buckets of soil for one week at 10 °C. These earthworms were randomly assigned to six 2.5 litre vessels, 0.18 m in depth. Three vessels were supplied with 4, and three supplied with 8 earthworms. The vessels contained 2 litres of soil, and were surface fed with excess paper pulp, obtained fresh from the mill or frozen for use at subsequent feeding. Sampling was every six weeks, except 8 weeks prior to termination of the experiment at 56 weeks. At each sampling, fresh soil and feed were supplied and earthworm masses were recorded. After the earthworms had matured, the discarded soil/feed was searched for cocoons by wet sieving through a mesh series of 6.7, 3.35 and 2 mm.
Experiment 2: *L. terrestris* hatchlings, produced from cocoons maintained at 20 °C (Butt et al. 1992) were transferred to water-filled Petri dishes maintained at 5 °C until 150 had been collected (this occurred within a time of 10 days). One hundred and thirty-five of these hatchlings (mean mass 53 mg) were randomly allocated to twenty-five pots. These consisted of five replicates of 1, 2, 4, 8 or 12 hatchlings per 0.3 litre pot. Each 4 cm deep pot was supplied with 200 g of moistened soil and a surface feed of 50 g of paper pulp mixed with 0.75 g of yeast extract, a proven feed for *L. terrestris* growth (Butt et al. 1992). Sampling occured after every 4 weeks at which time fresh feed was supplied, the earthworms were counted, assessed for sexual development and their mass recorded. The soil/feed matrix was completely replaced at every second sampling (i.e. every 8 weeks). If any of the earthworms were found to have reached sexual maturity the soil in which they had been maintained was searched for cocoons, either manually or by wet sieving when replaced.

Reproduction

Experiment 3: Using the formalin expellant method of Raw (1959), a large number of clitellate *L. terrestris* were collected from the grounds of the Open University. After washing in fresh water and storage for three days, in soil at 10 °C, 96 were selected for experimentation. All were apparently healthy, fully clitellate individuals with a mean mass of 5.7 g. These earthworms were randomly allocated to four replicates of treatments with 1, 2, 3, 4, 6 or 8 individuals per 0.6 litre pot. This gave initial mean densities of 9.5, 19, 28.5, 38, 57, and 76 g live mass l^{-1}. These pots, 0.04 m in depth, were supplied with 300 g of moistened soil and surface fed with separated cattle solids, previously dried to remove ammonia and then rewetted. This feed had proved successful in previous *L. terrestris* reproduction experiments (e.g. Butt 1991). Sampling was monthly, when soil and feed were replaced and the discarded medium searched for cocoons, as above. All earthworms had mass recorded and any mortality was noted, but replacement of dead individuals did not occur. The experiment was continued until zero cocoon production was recorded for all treatments.
Experiment 4: Mature (unmated) *L. terrestris* grown at 20 °C from isolated, laboratory produced hatchlings, were randomly allocated, in pairs, to pots of three sizes, 0.4, 0.75 and 1.2 litres with respective depths of 0.044, 0.076 and 0.115 m. With a mean mass of 5.2 g per earthworm, initial mean densities were 26.0, 13.9 and 8.7 g live mass l^{-1}. Eight replicates of each sized pot were supplied with moistened soil and surface fed with rewetted separated cattle solids. Pots were sampled every four weeks, when fresh soil and feed were supplied. A mid-sampling assessment was made and extra feed applied to the soil surface if required. At sampling, the soil/feed was searched for cocoons by wet sieving. All of the cocoons produced within each sized pot were incubated separately at 20 °C to determine their viability. Earthworm masses plus their general and reproductive condition were also recorded on sampling.

Results

Growth

Experiment 1: From figure 1 it can be shown that mean densities of 15 g live mass l^{-1} (4 per pot) and 22 g live mass l^{-1} (8 per pot) were attained after a period of 56 weeks. During this period zero mortality was recorded. The first clitellate individuals were recorder after

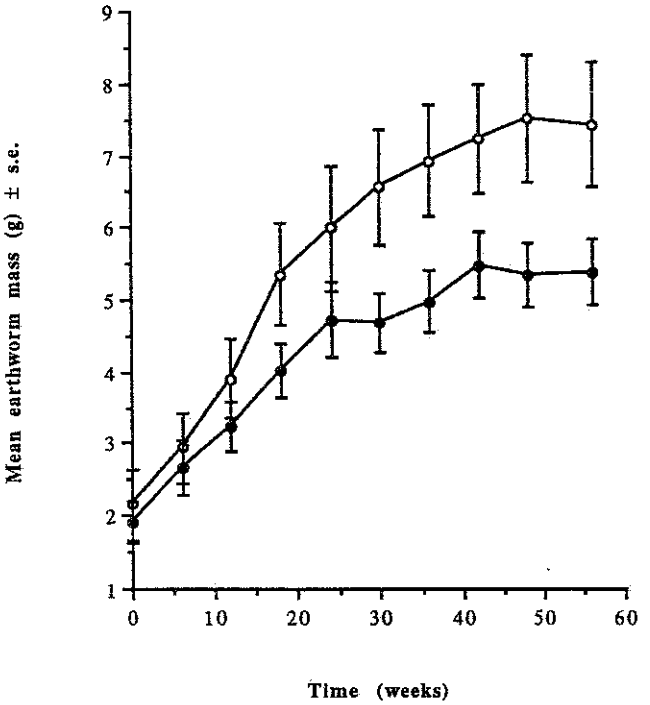

Fig. 1. Growth of field-collected, immature *L. terrestris* at two densities (○, n = 4; ●, n = 8) in pots with 2 litres of soil at 20 °C, fed with paper pulp

18 weeks at both densities. Cocoon production was first noted after 24 weeks (8 per pot) and 30 weeks (4 per pot) and continued thereafter until termination of the experiment. Viable cocoons were produced at both densities. All of the earthworms maintained at the lower density reached sexual maturity, whereas only 46% did so at the higher density. Earthworm growth rates at the two densities were significantly different ($p < 0.001$) between weeks 12–18.

Experiment 2: Figure 2 illustrates the growth of different numbers of hatchling *L. terrestris* in 0.3 litre pots. Significant differences ($p < 0.01$) in growth rates were obtained over the

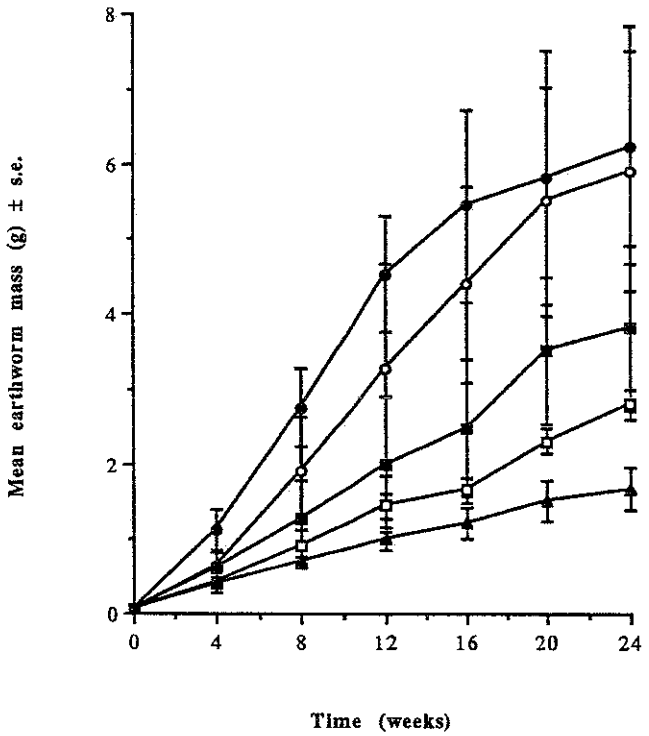

Fig. 2. Growth of hatchling *L. terrestris* at 5 densities (●, n = 1; ○, n = 2; ■, n = 4; □, n = 8; ▲, n = 12) in 0.3 litre pots at 20 °C fed with paper pulp and yeast extract

first 8 weeks of the experiment. After 24 weeks there were no significant differences in mean masses between pots containing 1 or 2 earthworms and between pots containing 4 or 8 earthworms. Clitellate individuals were first recorded after 12, 20 and 24 weeks in pots containing 1, 2, and 4 individuals respectively. Zero cocoon production was recorded during this experiment. In pots containing either 8 or 12 worms neither clitellum nor tubercula pubertatis development occurred. After 24 weeks the mean total mass per pot for each of the treatments was 6.2, 11.8, 15.2, 22.4 and 19.2 g live mass for 1, 2, 4, 8 and 12 earthworms per pot respectively. Zero mortality was recorded during this experiment.

Reproduction

Experiment 3: Table 1 shows reproductive success of *L. terrestris* at initial densities ranging from 9.5 to 76 g live mass l^{-1}. Initially all worms were clitellate but loss of reproductive condition and mortality increased with time at densities greater than 19 g live mass l^{-1} (2 worms per pot). Cocoon production was recorded at all but the highest density at the first sampling. The experiment was terminated after five months, when zero cocoon production was recorded for all treatments. For this whole period mean cocoon production was 1.5, 0.6, 0.1, 0.06, 0.04, and 0.0 cocoons per earthworm per month for 1, 2, 3, 4, 6 and 8 earthworms per pot respectively.

Experiment 4: Figure 3 represents mean monthly cocoon production over 24 weeks at three earthworm densities. Maximum production was 4.4 cocoons per earthworm per month in 0.75 litre pots between weeks 4 and 8 at a mean density of 14.9 g live mass l^{-1}. During the same period 2.4 and 3.6 cocoons were produced per earthworm per month in 0.4 and

Table 1. Mean cocoon production (*c*) (worm^{-1} month^{-1}), mean mass (*m*) (g) and mortality (%) of mature, field-collected *L. terrestris*, maintained at a range of densities in 0.6 litre pots. Initial mean mass = 5.7 g worm^{-1}. (na = not applicable due to high level of mortality)

No. worms per pot	Prodn. (*c*) Mass (*m*) Mortality (%)	Consecutive months				
		1	2	3	4	5
1	c	1.5	3.5	2.5	0.0	0.0
	m	6.1	6.6	5.8	5.9	5.8
	%	0	0	0	0	0
2	c	0.9	0.9	0.9	0.4	0.0
	m	6.2	6.3	6.4	6.5	5.6
	%	0	0	0	0	0
3	c	0.3	0.0	0.1	0.1	0.0
	m	5.9	5.7	5.3	4.9	na
	%	0	0	8	33	67
4	c	0.3	0.0	0.0	0.0	0.0
	m	5.7	5.3	4.8	na	na
	%	0	0	0	38	57
6	c	0.2	0.0	0.0	0.0	–
	m	5.2	4.3	3.7	na	0.0
	%	0	0	8	75	100
8	c	0.0	0.0	0.0	–	–
	m	4.8	3.9	3.0	0.0	0.0
	%	0	0	25	100	100

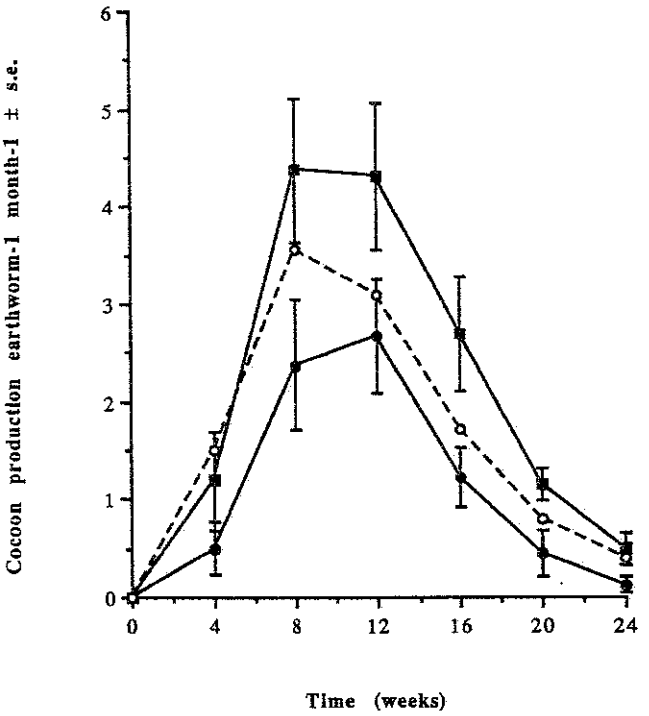

Fig. 3. Cocoon production of recently matured, paired *L. terrestris* fed with separated cattle solids at 20 °C, in culture vessels of three sizes (●, 0.4 l; ■, 0.75 l; ○, 1.2 l). Error bars omitted for 1.2 l pots

1.2 litre pots, at densities of 26.8 and 8.3 g live mass l^{-1} respectively. Error bars on figure 3 show significant differences ($p < 0.05$) between production figures in 0.4 and 0.75 litre pots after the initial 4 weeks. Production in 1.2 litre pots was not significantly different from production in each of the other two sizes of pot (error bars not shown for clarity).
Mean masses of all the experimental earthworms were greater than 5 g until sampling at 16 weeks when those in the largest pots had fallen to 4.5 g. At termination of the experiment mean masses were 4.4 g in the two smaller sized pots and 3.9 g in the largest pots. Zero mortality was recorded during the experiment at the higher densities but 50% of the earthworms in the 1.2 litre pots died between weeks 16–24. After 24 weeks loss of reproductive condition was recorded for 25, 19 and 37 percent of the surviving earthworms in the 0.4, 0.75 and 1.2 litre pots respectively. Overall viability of cocoons incubated at 20 °C was 67, 63 and 66 percent for 0.4, 0.75 and 1.2 litre pots respectively (total n = 406).

Discussion

Growth

The two growth experiments illustrate that an increased density of *L. terrestris* has a negative effect on growth rate and final mean earthworm mass. In both cases a larger density of earthworms resulted in a greater total mass per unit volume but individual masses were significantly reduced. The development of full reproductive capacity was also impaired with a larger number of earthworms per unit volume. Within the 2 litre system of experiment 1, an optimum number of individuals would probably lie somewhere between the two experimental treatments of 4 and 8, suggesting a mass in the range of 15 to 22 live g $litre^{-1}$. The observed density dependent effects were not a function of food deprivation as excess feed was provided. In the smaller 0.3 litre pots, utilising a superior feed (Butt et al. 1992), densities up to 40 g $litre^{-1}$ of mature earthworms (2 per pot) were attained although maturation was delayed by eight weeks (2 sampling periods) compared with animals grown in isolation. An optimal density for this system would therefore be 20–40 g

litre^{-1}. Hartenstein & Amico (1983) found that about 31 g live weight (8 worms) was the optimal biomass for growth of this species in 1.4 litre vessels with a substrate of 2:1, sewage sludge: soil. At 22 g live mass litre^{-1} this figure is in close accordance with the data obtained here. However, it is not clear from the earlier study whether the experimental earthworms were grown to maturity.
In the second growth experiment, single worms did not produce cocoons as *L. terrestris* is not parthenogenetic and groups of earthworms which matured also failed to reproduce as time for this was not allowed before the experiment was terminated.

Reproduction

Cocoon production was expected in all treatments of experiment 3, including single earthworm treatments of the field-collected animals, due to sperm storage from previous matings. As no cocoons were produced on first sampling at densities greater than 76 g litre^{-1}, it was inferred that this density must be in excess of the carying capacity for this species, in reproductive condition, under the given experimental design. Loss of mass and minimal reproduction linked with increasing mortality in subsequent months indicated that a population of *L. terrestris* could not be sustained at densities in excess of 19 g live mass per litre. It is possible that no mating took place during this experiment.
In experiment 4 mating must have occurred. Reproductive output by paired *L. terrestris* decreased significantly as density increased from 14.9 g litre^{-1} to 26.8 g litre^{-1}. However, pairs of this species initially at 8.3 g per litre (least crowded) produced less cocoons than those at almost double this density. Clearly factors other than density alone must have been involved as half of the earthworms at the lowest density treatment died during the experimental period. A steep reduction in cocoon production after 12 weeks appears to demonstrate reproductive fatigue at all densities for this species, under the given conditions. However, the use of recently matured, paired earthworms meant that lack of a mate or an inability to reproduce due to this temperature-related fatigue (Lofs-Holmin 1985; Butt 1991) was initially avoided.
Although cocoon production was reduced at certain densities the viability of those cocoons remained constant at approximately 65%. This suggests that reproductive effort may be adversely affected by density only in terms of the number of cocoons produced. This strategy appears to differ from *E. fetida* where the proportion of cocoons produced which hatch, has been shown to decline dramatically with increased density (Tomlin & Miller 1980).

General

The results from this series of experiments suggest that a production system for *L. terrestris* could be achieved within relatively small volumes. Some 15–25 g live mass can be accommodated per litre of soil, (equivalent to 3–5 adults). However, these experiments may not give a true picture of potential growth and reproduction in a larger system. Periodic disturbances caused by sampling and zero recruitment due to cocoon removal meant that dynamic populations were not permitted to develop. Growing hatchlings may increase the space requirement for reproductively active adults and have related impacts on feed requirements. Further work, employing a standard feedstock at a slightly lower temperature (Butt 1991) needs to be conducted with provision for enhanced population development in acceptable soil volumes, which may only require a depth of approximately 0.1 m.

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